Correlation Among PR, ER, PRL, CA15-3, CEA, LDH, and B2-M In Normal and Breast Cancer Women In Erbil-Kurdistan Region-Iraq

Mohammed Sharif H. Sulaiman[1], Sevan Omer Majed[2] and Fairuz Abdulla Kakasur[3]

[1]Department of Biology, College of Education-Akre, University of Dohuk
[2]Department of Biology, College of Education, Salahaddin University-Erbil
[3]Histopathology department, Rizgary Teaching Hospital, Ministry of Health,

Abstract --- **Breast cancer is a severely aggressive disease in women worldwide and its diagnosis at the early stage gives her a great option for treatment. Despite physical examination for detecting the breast tumors, biomarkers are helpful for treatment and identification. In this study, hormones (such as progesterone (PR), estrogen (ER), prolactin (PRL)) and proteins (CA 15-3, lactate dehydrogenase (LDH), β-2-microglobulin (β2-M)) were estimated in serum of 80 paired samples of normal and breast cancer women using Cobas 6000. The *Pvalue* ($p \leq 0.05$) was used to statistical significance. In normal cases, age factor was negatively correlated with PR (r2=-0.5) and positively correlated with ER (r2=0.5). The serum CEA level was negatively linked to ER(r2=0.5), but positively linked to PRL(r2=0.5) and CA 15-3 (r2=0.8). Moreover, the serum CA 15-3 level had a negative correlation with ER(r2=-0.6) and a positive correlation with PRL(r2=0.5). Serum PRL was positively related with ER(r2=0.6), but serum ER level had a negative correlation with PR(r2=-0.4). However, the age factor in patient with breast cancer was negatively correlated with the serum ER level(r2=-0.5), and positively correlated with serum CA 15-3(r2=0.4) and β2-M(r2=0.5). The serum β2-M level had a positive correlation with each of PRL(r2=0.4), CA 15-3(r2=0.7), CEA(r2=0.5), and LDH(r2=0.6). In addition, the serum LDH level had a positive correlation with each of PRL(r2=0.5), CA 15-3(r2=0.8), and CEA(r2=0.5). The serum CEA level was positively related to CA 15-3(r2=0.7), while the serum CA 15-3 was positively correlated with PR(r2=0.5). These results showed evidences that this strategy may be promising for breast cancer early diagnosis and treatment.**

Keywords*: Breast cancer, *Biomarker, PR, ER, PRL, CA 15-3, LDH and β2-M

I. INTRODUCTION

Despite the decrease in mortality rate of breast cancer, breast cancer is one of the precarious cancer in women over the past several decades (Majed and Mustafa, 2021). The early detection and identification of this form of cancer gives a woman more alternatives when it comes to treatment options. Although physical examination breast malignancy, such as mammography and ultrasonography, is believed to be the best procedure for diagnosing the breast tumor, there are growing worries about the possibility of cancer overdiagnosis (Welch et al., 2016). Serum biomarkers, such as hormones and proteins, are used for diagnosing and treating breast cancer at early stage (Robles-Frías et al., 2005). The staging system of TNM (tumor, nodes, and metastasis) has been a great classification developed by the American Joint Committee on Cancer (AJCC). The presence of axillary lymph node metastases is reported to be related to tumor size (Majed and Mustafa, 2021).

Hormones of progesterone (PR) and estrogen (ER) are the potential biomarkers for early diagnosis of breast cancer. In normal breast, progesterone plays a key role in controlling the lobular growth and estrogen can control the duct growth (Allison et al., 2020b). According to a new study, their receptors are overexpressed in the majority of breast malignant cells, resulting in a steady increase in breast cancer risk (Lange and Yee, 2008). Another study showed that there are correlations between estrogen receptor, progesterone receptor, and patient features in human breast cancer (Allison et al., 2020a).

Prolactin (PRL) is another hormone that is secreted by the cells of lactotroph of the front lobe of the pituitary gland. It is also generated by other tissues, for instance, lymphocytes, the mammary gland, placental decidua, prostate, and uterus (Robles-Frías et al., 2005). The biological functions of PRL are mediated via prolactin receptors. PRL serves as also stimulator for epithelial cell proliferation, DNA synthesis, and breast milk production (Robles-Frías et al., 2005, Rahman et al., 2014). The rising epidemiological and experimental proof indicates the function of PRL in breast tumorigenesis. It is alike to the development of normal mammary gland. The process of breast carcinogenesis is usually hormonally dependent (Radke et al., 2006, Radke et al., 2017, Kavarthapu et al., 2021).

The protein CA15-3 is an excellent biomarker and a type of a glycoprotein that belongs to a wide group of proteins. This protein is encoded by the MUC1 gene, which is elevated prior to surgery. CA15-3 is a breast cancer prognostic and tumor burden factor that is unaffected by other factors. For instance, they could be utilized in clinical contexts to define adjuvant treatment and predict patient outcomes (Zhao et al., 2021). CA15-3 can be used in conjunction with a physical examination, diagnostic imaging, and a patient's medical history. The CA15-3 test is particularly useful for determining the recurrence of illness and response to treatment (Li et al., 2020).

β2-microglobulin (β2-M) is a nonglycosylated protein. Expression level of this can promotes the growth and survival of cancer cells via the stimulation of vascular endothelial growth factor (VEGF) signaling pathway, protein kinase A

DOI: http://doi.org/10.24086/biohs2022/paper.710

(PKA)/cAMP-responsive element binding protein (CREB). It was also found to play a key role in cancer cell survival through the pathways of extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/Akt (Li et al., 2014, Wang et al., 2021). Previous studies showed that its overexpression levels are recorded in different cancers, including breast cancer, liver cancer, gastrointestinal cancers, prostate cancer, renal cell carcinoma, small cell lung cancer, and thyroid cancer (Josson et al., 2011).

Lactate dehydrogenase (LDH) in serum is found to be involved in performing anaerobic glycolysis. A previous study revealed that a significant increase in the number of malignant cells is influenced by the increased level of LDH in the cytosol of cells. This in breast cancer patients results in a significant increase in serum LDH levels (Said, 2019). The elevated level of LDH satisfies the malignant cells' need for anaerobic and metabolic glycolysis. The level of serum LDH in breast cancer patients is shown to be very high. This is similar to clinical TNM staging (Jia et al., 2018). The increased level of LDH reveals these changes and suggests that tumor angiogenesis, tumor growth circumstances, and tumor burden are all becoming more problematic. All of this contributes to the poor prognosis of cancer patients (Pelizzari et al., 2018).

The objective of this study is to find the correlation coefficient among PR, ER, PRL, CA15-3, LDH and β2-M in normal and breast cancer women.

II. MATERIALS AND METHODS

A. Sample collection

The present research is a type of prospective research. The samples were taken from individuals who attended the private BIO laboratory. Eighty paired blood samples from breast cancer cases and normal cases, who were not exposed to chemotherapy, hormonal therapy and radiation. All research cases obtained written informed consent and approval for publication. The Human Research Ethics Committee (HREC) at Salahuddin University-Erbil had previously approved all procedures used during the study (Reference No. 4d/132).

B. Concentration level measurement of the hormonal levels, and protein levels

The concentration level of seven indicators; including hormones (Progesterone (PR), Estrogen (ER), Prolactin (PRL)) and proteins (Cancer antigen 15-3 (CA15-3), Carcinoembryonic antigen (CEA), Lactate Dehydrogenase (LDH), and Beta-2 microglobulin (β2-M)) was estimated at the private BIO laboratory. To obtain the accurate result, the concentration level of all the indicators were immediately measured after withdrawing the blood from the cases. Approximately 5 milliliters of blood were received and allowed to clot at ambient temperature for 10 minutes in hollow disposable centrifuge tubes. The samples were then centrifuged at 3000 xg for 3 minutes.

Before being analyzed for these parameters, the serum samples were kept and separated at a temperature of -20°C. By using Cobas 6000 analyzer, the serum of them was estimated. The kit was provided by Aviva systems biology, USA.

C. Statistical analysis

GraphPad Prism software, version 8.4, was applied to analyze data and find out coefficient of correlation among the indicators. The experiment's findings are displayed as mean standard deviations. A one-way ANOVA test was used to compare the parameters in the various study groups. The Pvalues ($P \leq 0.05$) were considered significant in this regard. To assess the correlation between the different parameters in each patient group, the person relationship coefficient (r) was used.

III. RESULTS

A. The role of PR, ER, PRL, CA15-3, LDH and β2-M in healthy women

The outcomes of the parameters (PR, ER, PRL, CA15-3, CEA, LDH and β2-M) in both healthy and unhealthy women were analyzed to determine the effect of the breast cancer on these parameters. It seems that a noticeable difference was found between cases with breast cancer and healthy cases in the expression level of all the hormonal levels and protein levels. Table 1 showed the coefficient correlations among the parameters for the breast. It seems that there was a negative relationship between PR and age, which was r^2= -0.5; whereas, this relationship between these variables was positive by r^2 = 0.5. Besides, a positive association between age and both LDH and β2-M levels in healthy cases was found to be r^2 = 3 and 2 respectively. However, it can be also seen that the coefficient correlation, which was r^2 = -0.5, between CEA and ER was similar with CEA and PRL; whilst, the coefficient correlation, which made up r^2 = 0.8, between CEA and CA 15-3 was significantly positive. Similarly, the coefficient correlation, which constituted r^2= -0.6, between CA 15-3 and ER was almost the same as observed between CA 15-3 and PRL. However, a significant correlation between ER and PRL was found, which made up r^2 = 0.6.

Table 1: Correlation among age, PR, ER, PRL, CA15-3, LDH, and β2-M in normal women, pvalue ≤0.05.

Factors	Age	β2-M	LDH	CEA	CA15-3	PRL	ER
PR (ng/mL)	**-0.5**	-0.2	-0.2	0.4	0.3	-0.1	**-0.4**
ER (pg/mL)	**0.5**	0.1	-0.1	**-0.5**	**-0.6**	**0.6**	
PRL (μg/L)	0.1	-0.02	-0.4	**-0.5**	**-0.5**		
CA 15-3 (U/ml)	-0.4	-0.4	0.1	**0.8**			
CEA (ng/mL)	-0.3	-0.3	0.2				
LDH (IU/L)	0.3	-0.2					
β2-M (mg/L)	0.2						

B. The role of PR, ER, PRL, CA15-3, LDH and β2-M in breast cancer women

Table 2 displayed the coefficient correlations among the parameters (PR, ER, PRL, CA15-3, CEA, LDH and β2-M) for the women with breast cancer. There was a positive correlation between age and β2-M in patients with breast cancer. The coefficient correlation was r2 = 0.5. Similarly, this association, which made up r2 =0.4, was found between age and CA15-3. It appears that the coefficient correlation, which makes up 0.2,

DOI: http://doi.org/10.24086/biohs2022/paper.710

between age and PRL in the patients diagnosed with breast cancer was similar to the correlation between age and LDH. It can be seen that there is a significant association, which constituted r = 5 between the age and content level of ER in health women; whereas, the coefficient correlation in the women with breast cancer was inversely significant by r2 = -5. Furthermore, the content level of ER and PRL have a positive relationship in healthy women. The coefficient correlation was r2 = 6. This relationship, that makes up r2 = -1, between them was inversely negative for women with breast cancer. On the other hand, a negative relationship between PRL and PROG in healthy cases were showing by r2= -0.1 in comparison to cases with breast cancer (r2= 0.3).

Interestingly, the concentration level of CA 15-3, CEA, PRL, LDH and β2-M in patients with breast cancer compared to healthy women was revealing a significant correlation in table 2. The correlation between CA 15-3 and CEA is most significantly positive in both healthy women and women with breast cancer. The coefficient correlation between them was made up 0.7 for healthy women, compared with 0.8 for women with breast cancer. The CA 15-3 and LDH in women diagnosed with breast cancer were significantly correlated by r2 = 0.8 compared to only -0.07 for healthy women. Similarly, the CA 15-3 and β2-M in women with breast cancer were significantly linked by r2 = 0.7 compared to -0.4 for normal women. Furthermore, there was a positive correlation between the CA 15-3 and prolactin in women diagnosed with breast cancer. The coefficient correlation was r2 =0.5. Likewise, the prolactin and LDH had the same coefficient correlation in women with breast cancer. However, the coefficient correlation between β2-M and LDH was r2 = 0.6 in comparison with the concentration level of β2-M and PRL, which constitute r2 = 0.4 for women with breast cancer. Table 3 shows the comparison of six biomarkers in women with breast cancer and controls using multiple comparisons of least significant difference (LSD). The results were statistically evaluated using one-way analysis of variance (ANOVA) and LSD tests. The findings revealed a in/significant correlation between biomarkers of PR, ER, PRL, CA15-3, LDH, and β2-M.

Table 2: Correlation among age, PR, ER, PRL, CA15-3, LDH, and β2-M in women with breast cancer, pvalue ≤0.0
≤0.0

Factors	Age	β2-M	LDH	CEA	CA 15-3	PRL	ER
PR (ng/mL)	0.1	0.05	0.1	0.1	0.2	0.3	-0.1
ER (pg/mL)	**-0.5**	-0.3	-0.1	-0.2	-0.3	-0.1	
PRL (µg/L)	0.2	**0.4**	**0.5**	0.1	**0.5**		
CA 15-3 (U/ml)	**0.4**	**0.7**	**0.8**	**0.7**			
CEA (ng/mL)	0.01	**0.5**	**0.5**				
LDH (IU/L)	0.2	**0.6**					
β2-M (mg/L)	**0.5**						

Table 3: Serum concentrations of hormones and proteins of (normal) and (breast cancer) groups

Factors	Control	Breast cancer	LSD
No	80	80	
PR (ng/mL)	0.65±0.75	2.70±3.24	0.3
ER (pg/mL)	120.61±72.31	232.10±110.03	16.14
PRL (µg/L)	18.67±93.41	33.13±95.28	2.1
CA 15-3 (U/ml)	11.35±2.94	395.01±116.84	3.81
CEA (ng/mL)	1.30±0.48	7.19±1.40	0.6
LDH (IU/L)	171.31±22.55	379.54±165.22	18.3
β2-M (mg/L)	0.74±0.35	3.63±2.26	0.4

Note: Each value represents mean ± SD values. Pvalue was considered significantly differences (P≤ 0.05). No: Number of cases. SD: Standard deviation. LSD: Least Significant Difference.

C. Comparison of PR, ER, PRL, CA15-3, CEA, LDH, and β2M between normal and breast cancer groups

A total of 160 females examined separately in this Study, 80 without evident breast disease and 80 were with breast cancer as shown in table 3. Concentrations of all parameters are significantly elevated comparing to normal group. This indicates that there is a highly extensive correlation between breast cancer and raised the parameters levels. Since the disparity of prolactin over the menstrual cycle is inconsequential compared to its level in breast cancer patients, menstrual cycle status has not been taken into account. A substantial difference was observed in the level of prolactin PR (2.70±3.24 ng/mL) whereas the range was 0.65±0.75 in healthy groups. Likewise, the results showed that ER levels were significantly and positively correlated with patients with breast cancer (LSD: 16.14). Additional clinical factors such as ER, PRL, CA 15-3, CEA, LDH and β2M can carry an indication that there is a weighty relationship in breast cancer with raised tested parameters.

DOI: http://doi.org/10.24086/biohs2022/paper.710

IV. DISCUSSION

Breast cancer is one of the most lethal diseases in women all over the world. It is regarded as the second-worst distortion (Hu et al., 2021). The previous studies show that hormones like estrogen (ER) and progesterone (PR) can affect certain forms of breast cancer. Cancer cells in the breast have receptors (proteins) that bind to serum ER and PR, allowing them to proliferate. Hormone therapy is found to be a treatment that prevents hormones from binding to these receptors (Treeck et al., 2020, Madak-Erdogan et al., 2019, Saha et al., 2019). Saha and his colleagues confirm that after menopause, higher levels of it in the serum have been related to a high risk of breast cancer in females (Saha et al., 2019). However, a new study showed that the amount of serum PR produced by a woman's ovaries has also been linked to her risk of breast cancer. Exposure to this hormone for a long time and/or at high levels has been associated to an elevated risk of breast cancer (Trabert et al., 2020).

However, a higher level of serum CA 15.3 suggests a bad outcome and a higher risk of occult disease. The higher the CA 15.3 level, the worse the prognosis in breast cancer patients. This is a concerning aspect of the first occurrence in patients with advanced breast cancer (Hing et al., 2020). A new study states that the presence of a greater amount of serum CA 15-3 differs from metastatic site than the primary tumor (Goodwin et al., 2021). Another study shows that the greater values of biomarker are observed in metastatic breast cancer patients, as compared to the primary breast cancer patients and also in contrast to people with greater values of biomarker before surgery (Ruswendro et al., 2021).

However, beta-2-microglobulin (β2-M) in breast cancer has been shown to operate as a growth factor and signaling molecule. The goal of the study is to investigate the mechanism of β2-M activity in breast cancer by characterizing β2-M expression in molecular subtypes of the disease (Schuh, 2019). Serum levels of β2-M have been found to be higher in breast cancer patients. Serum β2-M levels in cases with breast cancer were considerably greater than those in individuals with benign breast tumors, according to an immunohistochemistry (IHC). Furthermore, patients with the four molecular subtypes had significantly varied amounts of β2-M protein expression in breast cancer cells (Jongvilaikasem et al., 2021).

However, because serum biomarkers are relatively easy and inexpensive to assess, regular measurements of their values provide crucial information for earlier detection of recurrence of the breast cancer (Marques et al., 2018, Jain et al., 2021). The majority of studies that investigate the relationship between breast cancer risk and prolactin (PRL) expression level focus on circulating PRL levels. However, in addition to acting as an endocrine hormone, the serum PRL produced locally by mammary cells has an effect on autocrine cells or neighboring paracrine cells (Shams et al., 2021, Chen et al., 2021, Craig et al., 2021). The serum PRL hormone appears to have a significant role in the breast cancer progression by blocking apoptosis and increasing cell proliferation, according to previous study. Furthermore, this hormone promotes cell motility and angiogenesis and this is a common cause of cancer metastasis(Motamedi et al., 2020).

Another study shows that all of the verified studies were pooled to provide a huge sample size. This made it possible to obtain more consistent results with more statistical power. This method assisted in the discovery of a link between breast cancer risk and plasma prolactin levels. Further research revealed that, in addition to serving as a bio marker, prolactin may be a cause of breast cancer. Serum LDH levels were shown to be higher in patients with breast cancer, and this was linked to the size of the tumor and clinical stage (Wang et al., 2021). Other previous studies had come up with the same conclusions (Varma et al., 2021).

V- Conclusion

Levels of hormones (PR, ER, and PRL) and proteins (CA15-3, LDH, and β2-M) were positively or negatively correlated with breast cancer women. The high/low levels of them were also relatively correlated with each other. In addition, the high levels of them were linked with poorer overall survival result. These hormones and proteins may be used as biomarkers for diagnosing breast cancer at the early stage.

Acknowledgements

The authors would like to thank Dr. Fairuz of the Histopathology department, Rizgary Teaching Hospital for helping collecting a raw data.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Dr. Sevan conceived the study, developed the methodology, and provided software. Dr. Fairuz validated the data. Dr. Sevan performed formal analysis and performed investigations. Dr. Fairuz provided resources. Dr. Sevan and Mr. Mohammed Sharif wrote the original draft. Mr. Mohammed Sharif reviewed and edited the manuscript. Dr. Sevan and Mr. Mohammed Sharif visualized data and supervised the study. Dr. Sevan was the project administrator. The authenticity of all the raw data was confirmed by Dr. Fairuz and Dr. Sevan. All authors read and approved the final manuscript.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. ALLISON, K. H., HAMMOND, M. E. H., DOWSETT, M., MCKERNIN, S. E., CAREY, L. A., FITZGIBBONS, P. L., HAYES, D. F., LAKHANI, S. R., CHAVEZ-MACGREGOR, M. & PERLMUTTER, J. (2020a) Estrogen and progesterone receptor testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists guideline update. *Archives of pathology & laboratory medicine,* 144**,** 545-563.
2. ALLISON, K. H., HAMMOND, M. E. H., DOWSETT, M., MCKERNIN, S. E., CAREY, L. A., FITZGIBBONS, P. L., HAYES, D. F., LAKHANI, S. R., CHAVEZ-MACGREGOR, M. & PERLMUTTER, J. (2020b) Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update.
3. JIA, Z., ZHANG, J., WANG, Z., WANG, B., WANG, L., CAO, J., TAO, Z. & HU, X. (2018) An explorative analysis of the prognostic value of lactate dehydrogenase for survival and the chemotherapeutic response in patients with advanced triple-negative breast cancer. *Oncotarget,* 9**,** 10714.
4. JOSSON, S., NOMURA, T., LIN, J.-T., HUANG, W.-C., WU, D., ZHAU, H. E., ZAYZAFOON, M., WEIZMANN, M. N., GURURAJAN, M. & CHUNG, L. W. (2011) β2-microglobulin induces epithelial to mesenchymal transition and confers cancer lethality and bone metastasis in human cancer cells. *Cancer research,* 71**,** 2600-2610.
5. KAVARTHAPU, R., ANBAZHAGAN, R. & DUFAU, M. L. (2021) Crosstalk between PRLR and EGFR/HER2 Signaling Pathways in Breast Cancer. *Cancers,* 13**,** 4685.
6. LANGE, C. A. & YEE, D. (2008) Progesterone and breast cancer. *Women's health (London, England),* 4**,** 151-162. 10.2217/17455057.4.2.151.
7. LI, J., LIU, L., FENG, Z., WANG, X., HUANG, Y., DAI, H., ZHANG, L., SONG, F., WANG, D. & ZHANG, P. (2020) Tumor markers CA15-3, CA125, CEA and breast cancer survival by molecular subtype: a cohort study. *Breast Cancer***,** 1-10.
8. LI, K., DU, H., LIAN, X., YANG, S., CHAI, D., WANG, C., YANG, R. & CHEN, X. (2014) Characterization of β2-microglobulin expression in different types of breast cancer. *BMC cancer,* 14**,** 1-9.
9. MAJED, S. O. & MUSTAFA, S. A. (2021) MACE-Seq-based coding RNA and TrueQuant-based small RNA profile in breast cancer: tumor-suppressive miRNA-1275 identified as a novel marker. *BMC Cancer,* 21**,** 473. 10.1186/s12885-021-08218-4.
10. PELIZZARI, G., GERRATANA, L., BASILE, D., VITALE, M. G., BARTOLETTI, M., LISANTI, C., FANOTTO, V., LIGUORI, A., CINAUSERO, M. & BOZZA, C. (2018). Prognostic role of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in metastatic breast cancer (MBC) patients: First clues for cost-effective biomarkers. American Society of Clinical Oncology.
11. RADKE, I., GÖTTE, M., KERSTING, C., MATTSSON, B., KIESEL, L. & WÜLFING, P. (2006) Expression and prognostic impact of the protein tyrosine phosphatases PRL-1, PRL-2, and PRL-3 in breast cancer. *British journal of cancer,* 95**,** 347-354.
12. RADKE, I., GÖTTE, M., SMOLLICH, M., SCHARLE, N., KIESEL, L. & WÜLFING, P. (2017) Expression of PRL-3 regulates proliferation and invasion of breast cancer cells in vitro. *Archives of gynecology and obstetrics,* 296**,** 1153-1160.
13. RAHMAN, T., CLEVENGER, C. V., KAKLAMANI, V., LAURIELLO, J., CAMPBELL, A., MALWITZ, K. & KIRKLAND, R. S. (2014) Antipsychotic treatment in breast cancer patients. *American Journal of Psychiatry,* 171**,** 616-621.
14. ROBLES-FRÍAS, A., GONZÁLEZ-CÁMPORA, R., MARTÍNEZ-PARRA, D., ROBLES-FRÍAS, M. J., VÁZQUEZ-CEREZUELA, T., OTAL-SALAVERRI, C. & FERNÁNDEZ-PALACÍN, A. (2005) Robinson cytologic grading of invasive ductal breast carcinoma: correlation with histologic grading and regional lymph node metastasis. *Acta cytologica,* 49**,** 149-153.
15. SAID, N. M. (2019) Three gold indicators for breast cancer prognosis: a case–control study with ROC analysis for novel ratios related to CBC with (ALP and LDH). *Molecular biology reports,* 46**,** 2013-2027.
16. WANG, S., MA, L., WANG, Z., HE, H., CHEN, H., DUAN, Z., LI, Y., SI, Q., CHUANG, T.-H. & CHEN, C. (2021) Lactate Dehydrogenase-A (LDH-A) Preserves Cancer Stemness and Recruitment of Tumor-Associated Macrophages to Promote Breast Cancer Progression. *Frontiers in Oncology,* 11**,** 2032.
17. WELCH, H. G., PROROK, P. C., O'MALLEY, A. J. & KRAMER, B. S. (2016) Breast-Cancer Tumor Size, Overdiagnosis, and Mammography Screening Effectiveness. *N Engl J Med,* 375**,** 1438-1447. 10.1056/NEJMoa1600249.
18. ZHAO, W., LI, X., WANG, W., CHEN, B., WANG, L., ZHANG, N., WANG, Z. & YANG, Q. (2021) Association of Preoperative Serum Levels of CEA and CA15-3 with Molecular Subtypes of Breast Cancer. *Disease Markers,* 2021.

DOI: http://doi.org/10.24086/biohs2022/paper.710